Environmental, epigenetic, and genetic interactions and human health protection

Shu-Li Julie Wang[1], Chung-Hsing Chen[2], Shih-Sheng Jiang[2], I-Shou Chang[2], Yuliang Leon Guo [1]

[1] National Institute of Environmental Health Sciences
[2]National Institute of Cancer Research

National Health Research Institutes, Zhunan, Taiwan

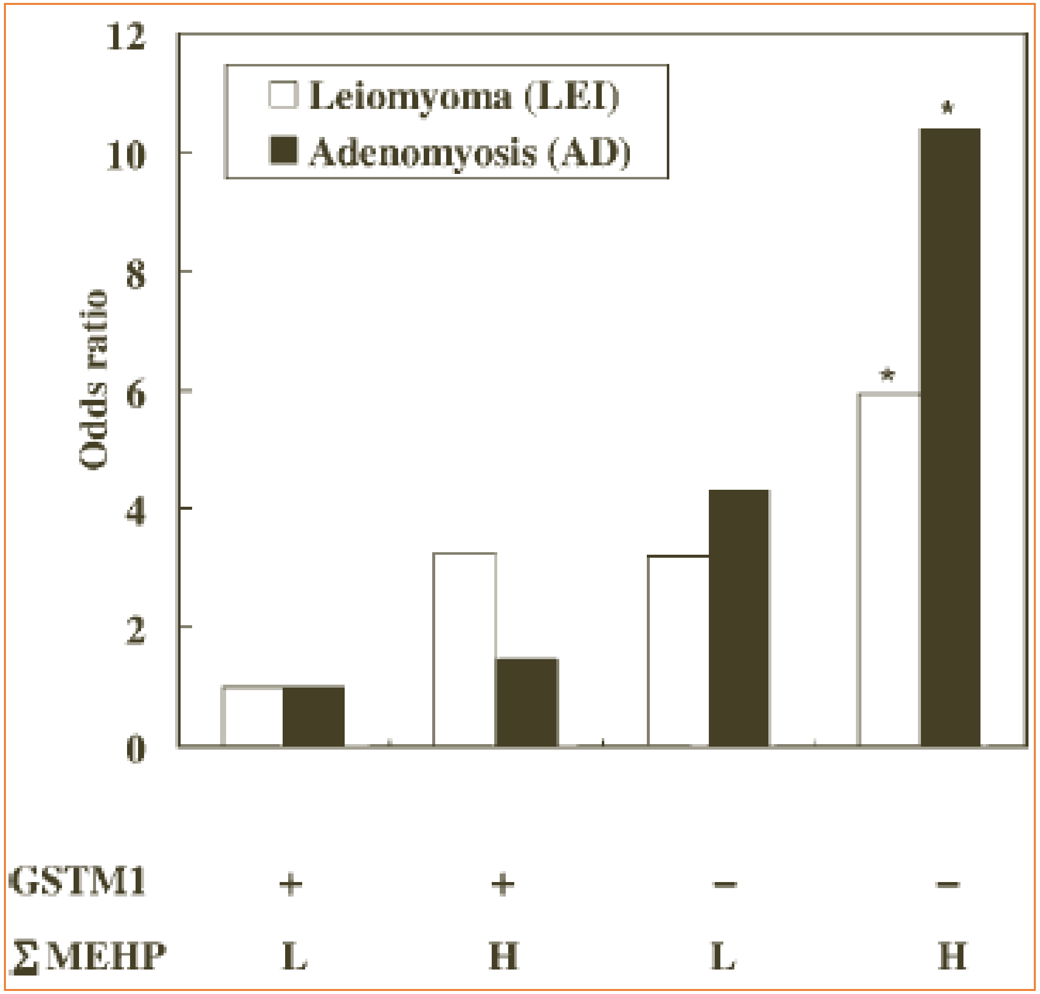
(slwang@nhri.org.tw)

Hohuan mountain (3151m), middle Taiwan

Rationale

- Human diseases: GxE
- Gene express differ by genotype
- DNA methylation: relatively stable control for gene expression (as compared histone modification and micro RNA
- Epigenome: Illumina 450k, 850k (Epic)
- Altered DNA methylation patterns by environmental factors
- Early days of lives exposure and DoHaD
- G x G x E

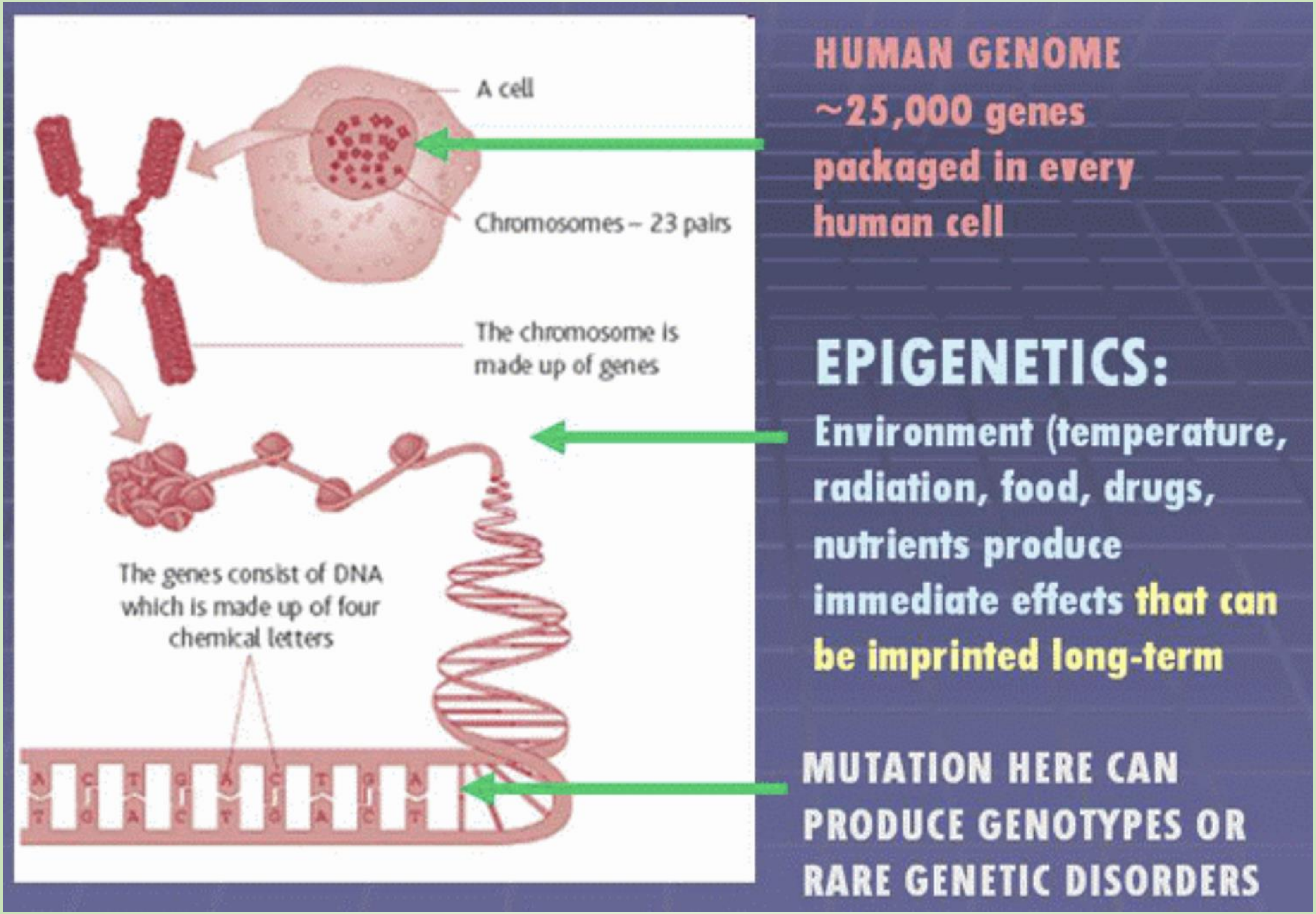
DEHP曝露及解毒基因
與子宮(腺)肌瘤風險(暴露與基因的交互作用)



Median MEHP (38.9 mg/g c).
Significant trend was found for AD ＆ LEI

Huang et al., Human Repro 2010

Epigentics

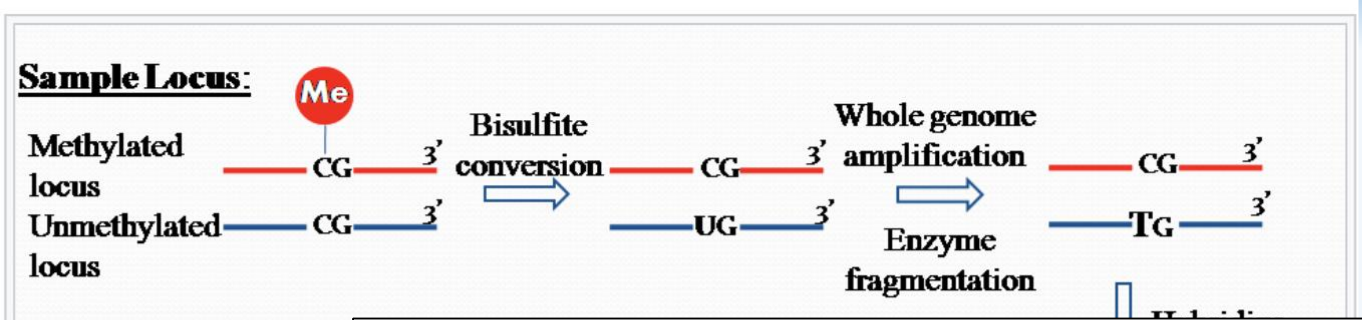


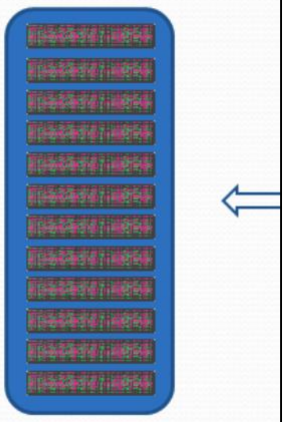
HUMAN GENOME
~25,000 genes packaged in every human cell

EPIGENETICS:

Environment (temperature, radiation, food, drugs, nutrients produce immediate effects that can be imprinted long-term

MUTATION HERE CAN PRODUCE GENOTYPES OR RARE GENETIC DISORDERS





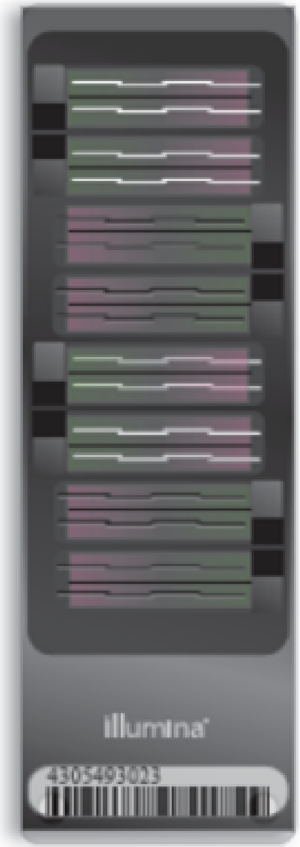
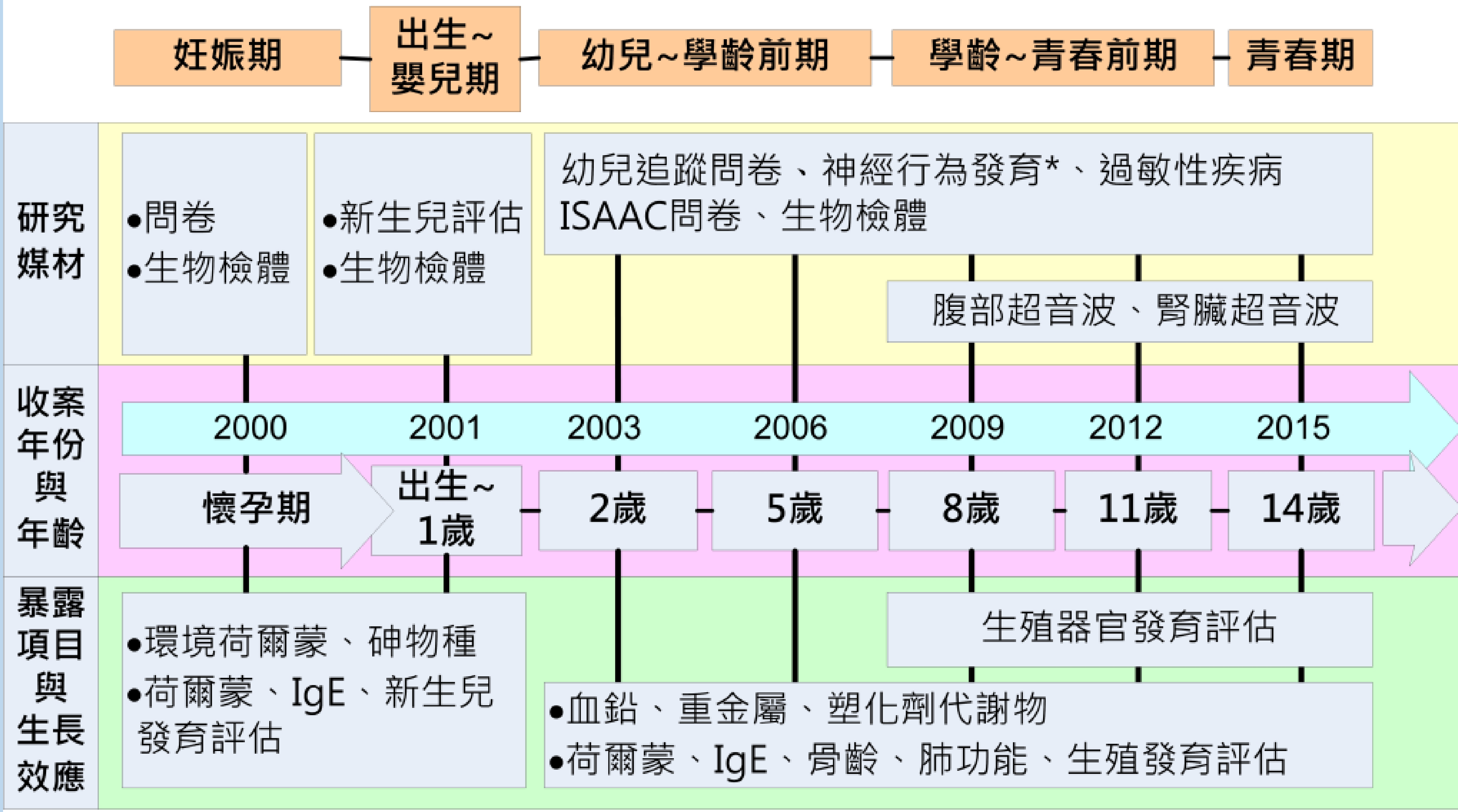


Figure 1: The Infinium MethylationEPIC BeadChip—The Infinium MethylationEPIC BeadChip features > 850,000 CpGs in enhancer regions, gene bodies, promoters, and CpG islands.

METHODS

- 15-year follow-up birth cohort study:
  - prenatal exposure to phthalate plasticizer associated with postnatal health effects
    - decreased sex hormone levels
    - reduced reproductive organ growth
    - increased behavioral problems
    - decreased intelligent quotients
    - allergic symptoms
- Archived cord blood DNA samples from 64 mother-children pairs
  - DNA methylation levels at more than 450,000 CpG sites measured using Illumina Infinium HumanMethylation450 BeadChips.

台灣孕婦與新生兒世代研究 – 前驅試驗研究 (TMICS-pilot)



妊娠期 – 出生~嬰兒期 – 幼兒~學齡前期 – 學齡~青春前期 – 青春期

研究媒材

- 問卷
- 生物檢體

- 新生兒評估
- 生物檢體

幼兒追蹤問卷、神經行為發育*、過敏性疾病
ISAAC問卷、生物檢體

腹部超音波、腎臟超音波

收案年份與年齡

2000 2001 2003 2006 2009 2012 2015

懷孕期 – 出生~1歲 – 2歲 – 5歲 – 8歲 – 11歲 – 14歲

暴露項目與生長效應

- 環境荷爾蒙、砷物種
- 荷爾蒙、IgE、新生兒發育評估

生殖器官發育評估

- 血鉛、重金屬、塑化劑代謝物
- 荷爾蒙、IgE、骨齡、肺功能、生殖發育評估

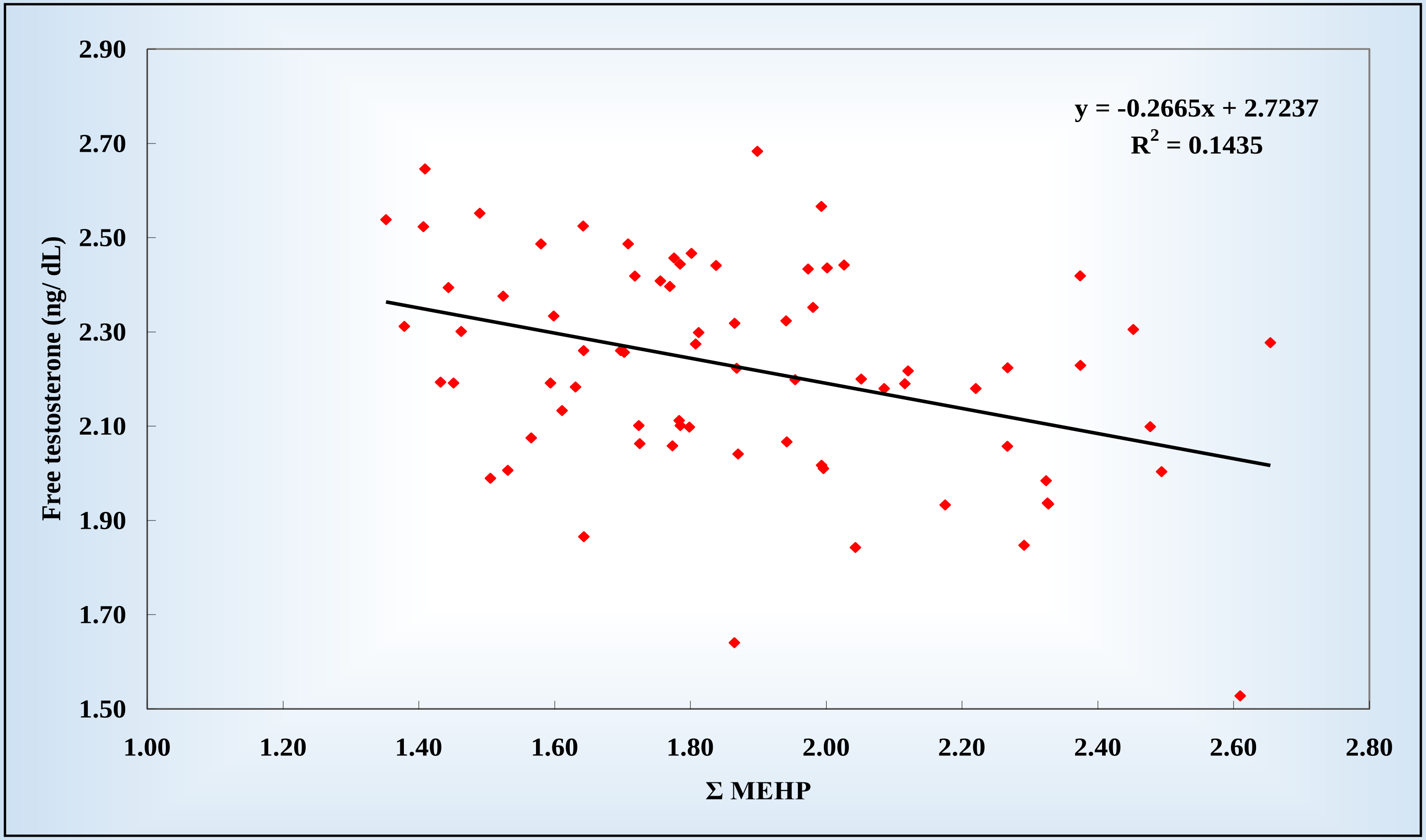
*兒童神經行為發育量測工具，包括：CCDI：學齡前兒童行為問卷；PSAI；學齡前兒童日常問卷；CBCL：4-16歲行為檢核表，魏式智力量表，氣質量表

生物檢體：孕產婦的靜脈血、尿液、胎盤、臍帶血、母乳，兒童的尿液、靜脈血、頭髮

11-year follow-up

內分泌、生殖發育評估

Decreased Free TT according to Maternal U-MEHP in Female Newborns



Lin LC et al., Chemosphere 2011

Urinary phthalate metabolite concentrations (μg/g creatinine) & serum IgE levels (IU/ml) in 8-year-old children

Phthalate metabolites	Maternal urinary phthalate [a]		Children's urinary phthalate at 8 yr[a]	
	Allergic children[b] (n=72)	Non-allergic children (n=58)	Allergic children[b] (n=83)	Non-allergic children (n=76)
MEHP	**0.50 (0.02)***	0.24 (0.26)	0.16 (0.34)	0.02 (0.87)
ΣDEHP	0.20 (0.32)	0.12 (0.51)	0.21 (0.28)	0.11 (0.53)
MBzP	0.02 (0.94)	0.22 (0.39)	0.01 (0.98)	**0.32 (0.02)***
MEP	0.11 (0.55)	**0.43 (0.09)#**	0.07 (0.65)	0.20 (0.24)

All data are presented as unstandardized regression coefficient (B) and *p*-value.

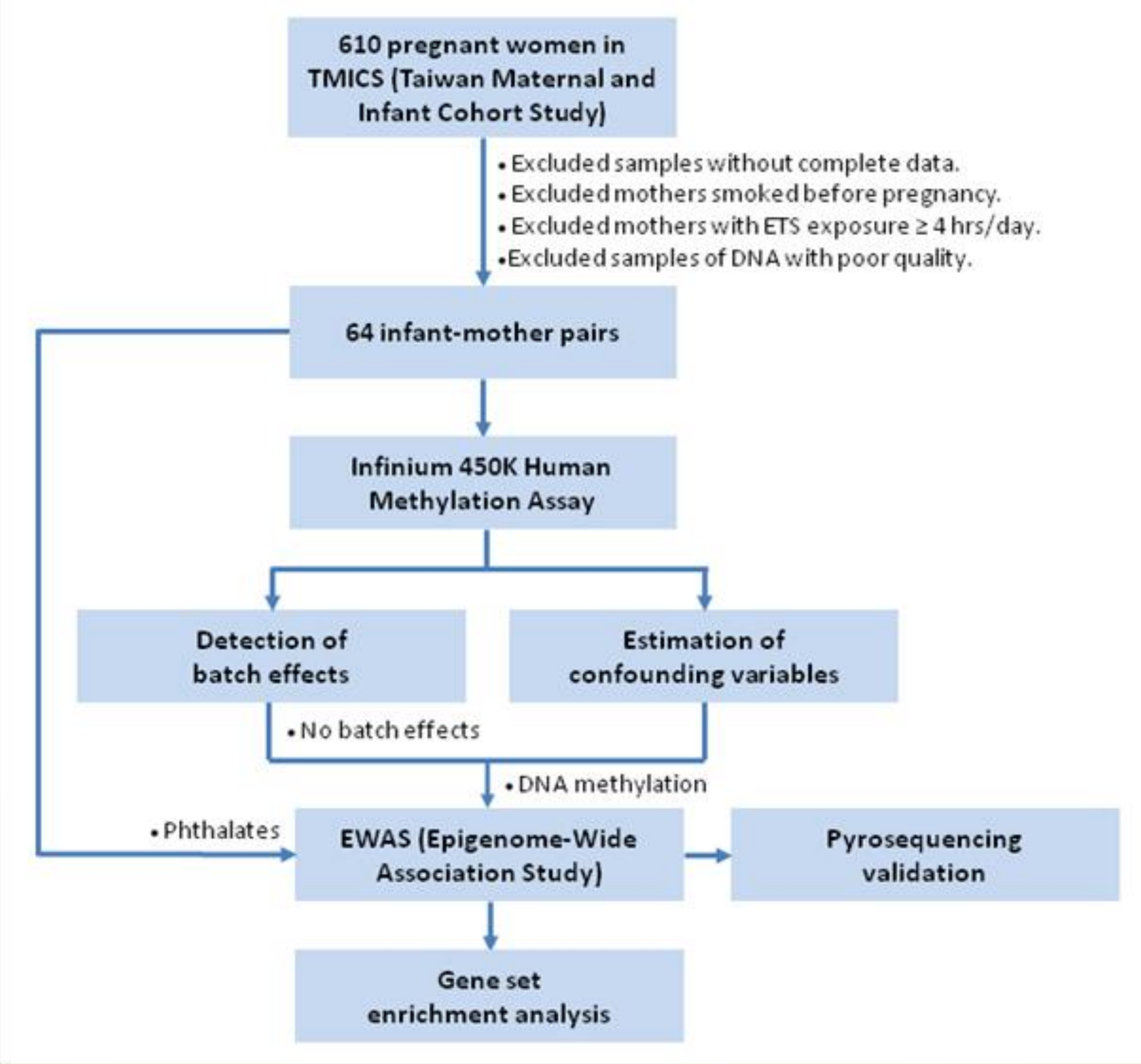
[a] Adjusted for gender, and maternal and paternal allergies.

[b] Children with any kind of allergies indicate children have any allergy including asthma, dermatitis, rhinitis and conjunctivitis.

* $p<0.05$, # $p<0.10$. ΣDEHP: MEHP+MEHHP+MEOHP

Ku et al. 2014

DNA methylation investigation



610 pregnant women in TMICS (Taiwan Maternal and Infant Cohort Study)

- Excluded samples without complete data.
- Excluded mothers smoked before pregnancy.
- Excluded mothers with ETS exposure ≥ 4 hrs/day.
- Excluded samples of DNA with poor quality.

64 infant-mother pairs

Infinium 450K Human Methylation Assay

Detection of batch effects

Estimation of confounding variables

- No batch effects

- DNA methylation

- Phthalates

EWAS (Epigenome-Wide Association Study)

Pyrosequencing validation

Gene set enrichment analysis

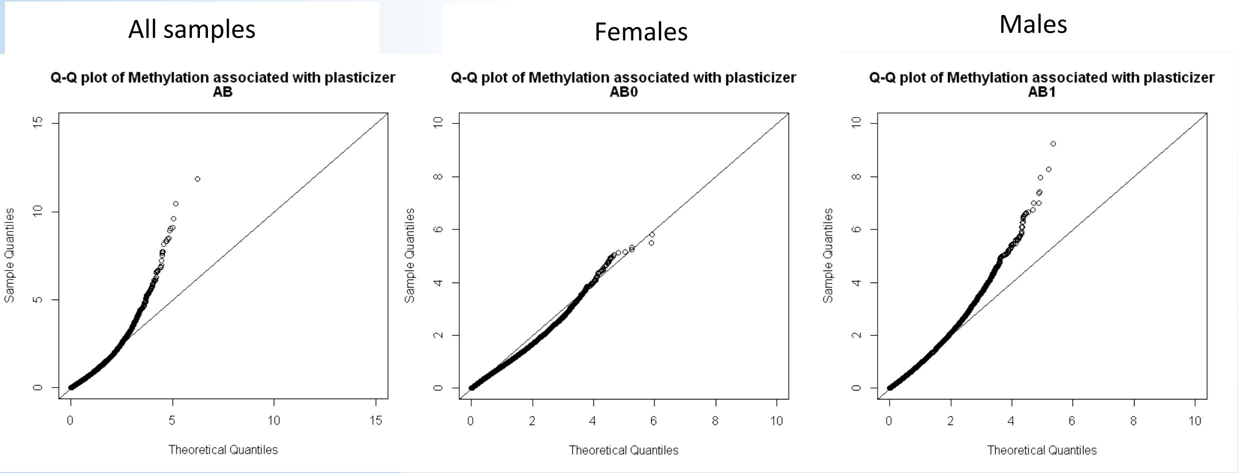
Altered DNA methylation according to Phthalates

- We consider the linear regression

$$\log(Methy/Unmethy) = SV + batch + gender + BS + MEOHP$$

where SV denote the surrogate variable and BS denote the bisulfite conversion efficiency.

- We plot Q-Q (Quantile-Quantile) plots for p-value of $MEOHP$ over all 62 samples, 24 female samples and 38 male samples.



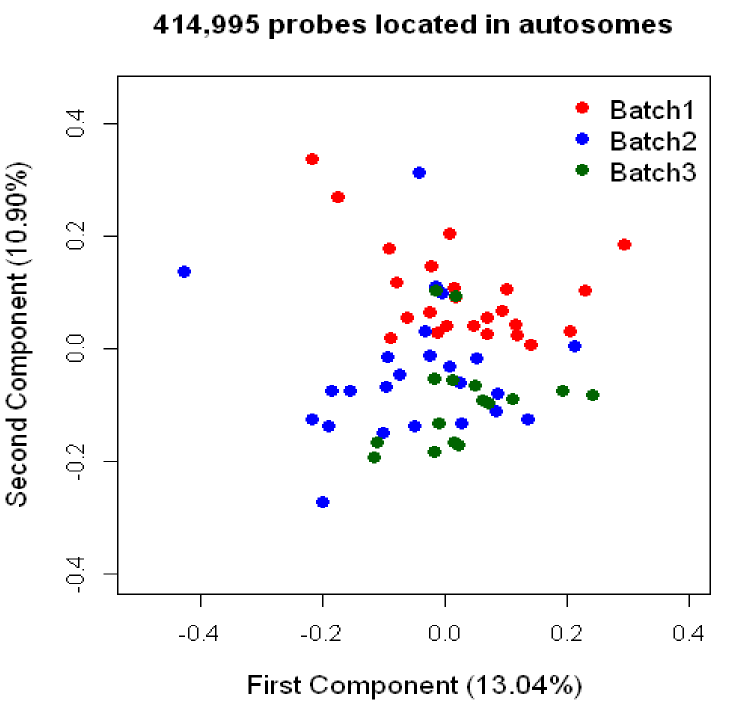
All samples

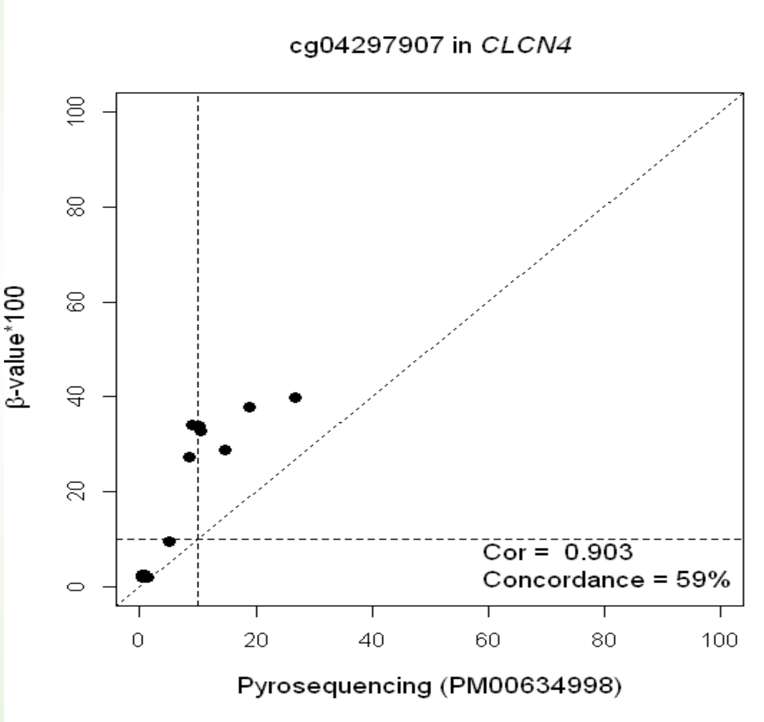
Females

Males

Chen et al.

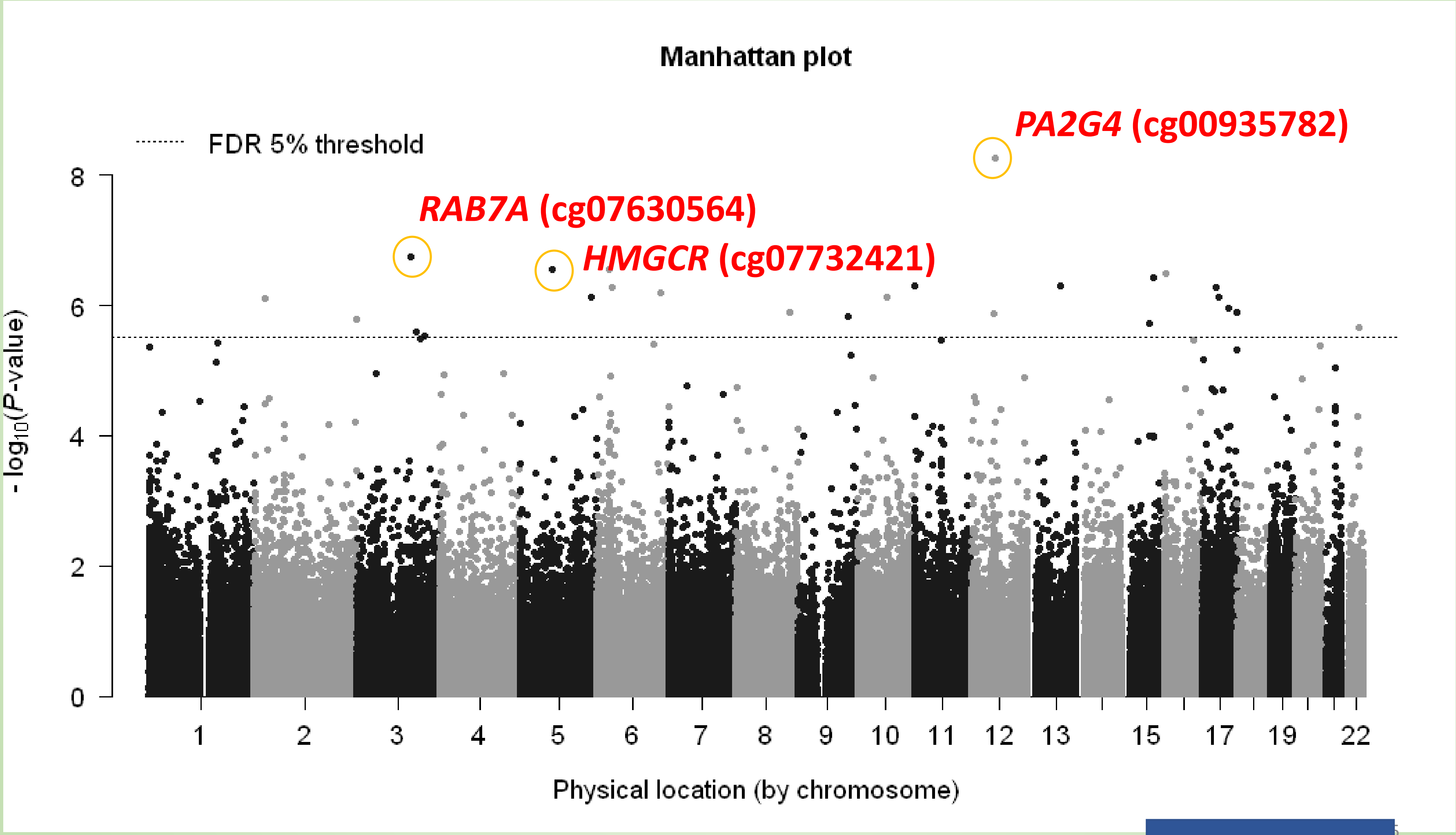
Methods


414,995 probes located in autosomes
Batch1
Batch2
Batch3
Second Component (10.90%)
First Component (13.04%)
-0.4
-0.2
0.0
0.2
0.4


cg04297907 in *CLCN4*
β-value*100
Pyrosequencing (PM00634998)
0
20
40
60
80
100
Cor = 0.903
Concordance = 59%

Result

Top 3 genes reported in the EWAS (Epigenome-Wide Association Study)



***PA2G4* (cg00935782)**

***RAB7A* (cg07630564)**

***HMGCR* (cg07732421)**

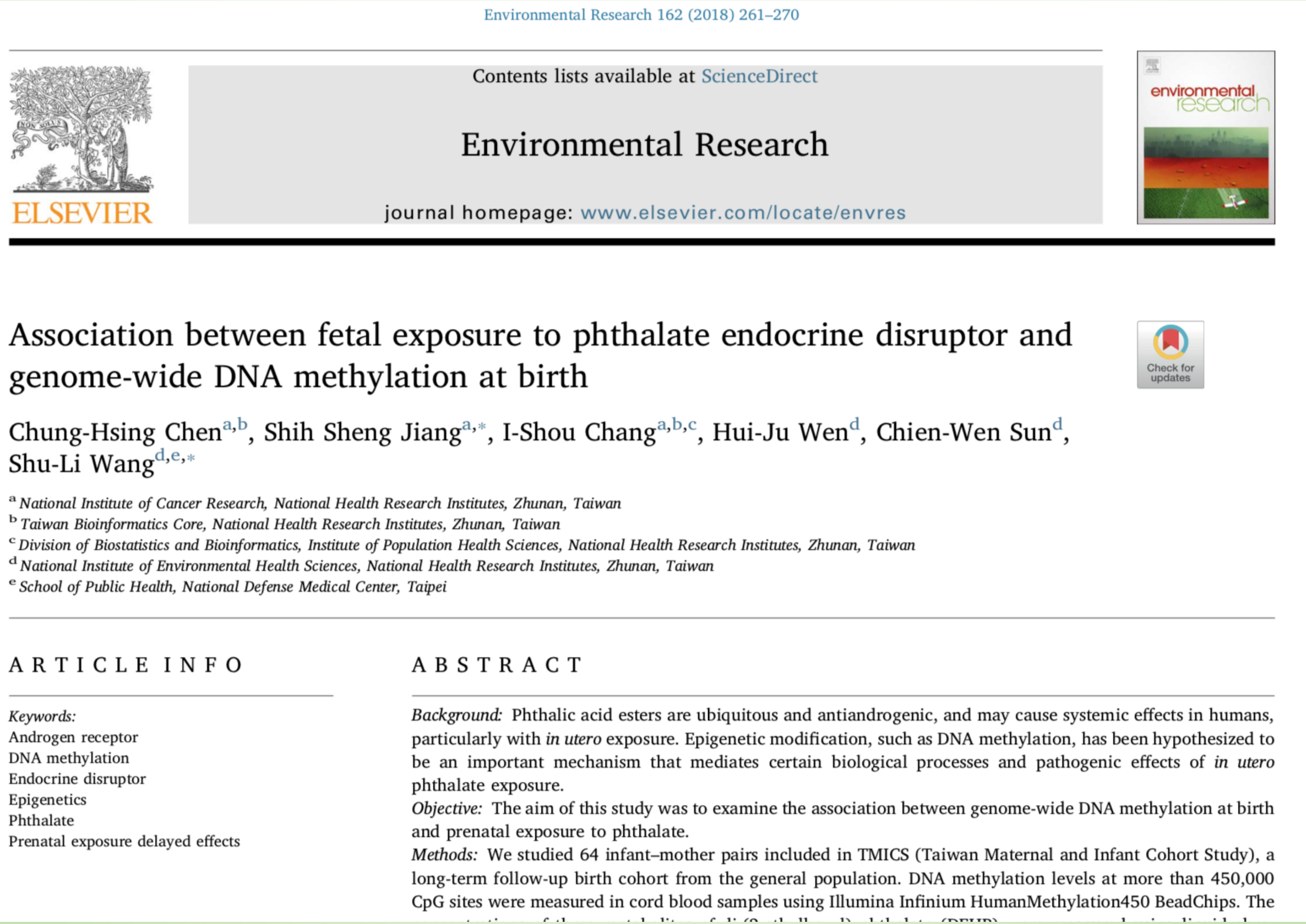
Pathway analysis result

Gene set	NOM P-val^{c}	FDR q-val^{d}
HALLMARK_WNT_BETA_CATENIN_SIGNALING	0	0.003
HALLMARK_PEROXISOME	0.002	0.006
HALLMARK_MITOTIC_SPINDLE	0	0.012
HALLMARK_HEME_METABOLISM	0	0.010
HALLMARK_TGF_BETA_SIGNALING	0.005	0.026
HALLMARK_NOTCH_SIGNALING	0.014	0.034
HALLMARK_CHOLESTEROL_HOMEOSTASIS	0.016	0.056
HALLMARK_P53_PATHWAY	0.021	0.084
HALLMARK_ESTROGEN_RESPONSE_LATE	0.026	0.105
HALLMARK_ESTROGEN_RESPONSE_EARLY	0.029	0.104
HALLMARK_UV_RESPONSE_DN	0.038	0.108
HALLMARK_BILE_ACID_METABOLISM	0.065	0.140
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.056	0.130
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.063	0.124
HALLMARK_GLYCOLYSIS	0.072	0.132
HALLMARK_IL2_STAT5_SIGNALING	0.087	0.173
HALLMARK_E2F_TARGETS	0.016	0.201
HALLMARK_MYC_TARGETS_V2	0.057	0.201
HALLMARK_PROTEIN_SECRETION	0.05	0.255
HALLMARK_SPERMATOGENESIS	0.137	0.259
HALLMARK_ANDROGEN_RESPONSE	0.019	0.292
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.198	0.293

Results

- 25 CpG sites whose methylation levels in cord blood significantly correlated with prenatal DEHP exposure
- Significant enrichment of genes showing DNA methylation changes in response to exposure involved in:
  - Androgen response,
  - Estrogen response, and
  - Spermatogenesis
- PA2G4, HMGCR, and XRCC6 genes were involved in genes in response to androgen
- Significant altered DNA methylation sites is related to immune function

DNA methylation in fetus of phthalate exposure



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Association between fetal exposure to phthalate endocrine disruptor and genome-wide DNA methylation at birth

Chung-Hsing Chen[a,b], Shih Sheng Jiang[a,*], I-Shou Chang[a,b,c], Hui-Ju Wen[d], Chien-Wen Sun[d], Shu-Li Wang[d,e,*]

[a] National Institute of Cancer Research, National Health Research Institutes, Zhunan, Taiwan
[b] Taiwan Bioinformatics Core, National Health Research Institutes, Zhunan, Taiwan
[c] Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan
[d] National Institute of Environmental Health Sciences, National Health Research Institutes, Zhunan, Taiwan
[e] School of Public Health, National Defense Medical Center, Taipei

ARTICLE INFO

Keywords:
Androgen receptor
DNA methylation
Endocrine disruptor
Epigenetics
Phthalate
Prenatal exposure delayed effects

ABSTRACT

Background: Phthalic acid esters are ubiquitous and antiandrogenic, and may cause systemic effects in humans, particularly with *in utero* exposure. Epigenetic modification, such as DNA methylation, has been hypothesized to be an important mechanism that mediates certain biological processes and pathogenic effects of *in utero* phthalate exposure.

Objective: The aim of this study was to examine the association between genome-wide DNA methylation at birth and prenatal exposure to phthalate.

Methods: We studied 64 infant–mother pairs included in TMICS (Taiwan Maternal and Infant Cohort Study), a long-term follow-up birth cohort from the general population. DNA methylation levels at more than 450,000 CpG sites were measured in cord blood samples using Illumina Infinium HumanMethylation450 BeadChips. The

Arsenic is everywhere

Juice

Rice

Possible Sources:
Fertilizer/Herbicides/Soil/Water

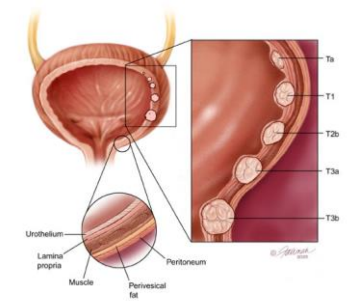
FDA, July & September,2013

Health effects of Arsenic

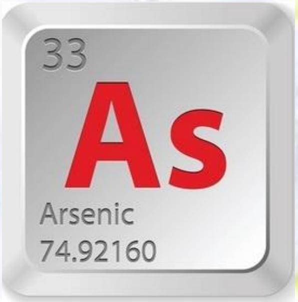
Skin cancer

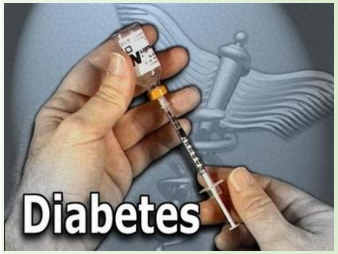
Peripheral vascular disease

Bladder cancer

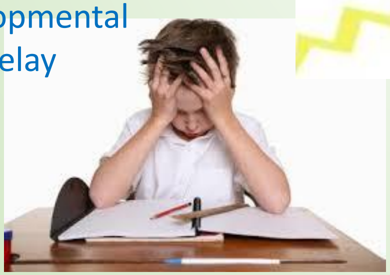


Lung cancer





Developmental delay



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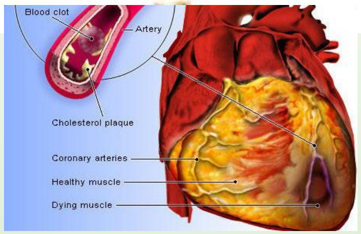
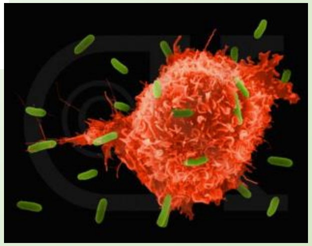
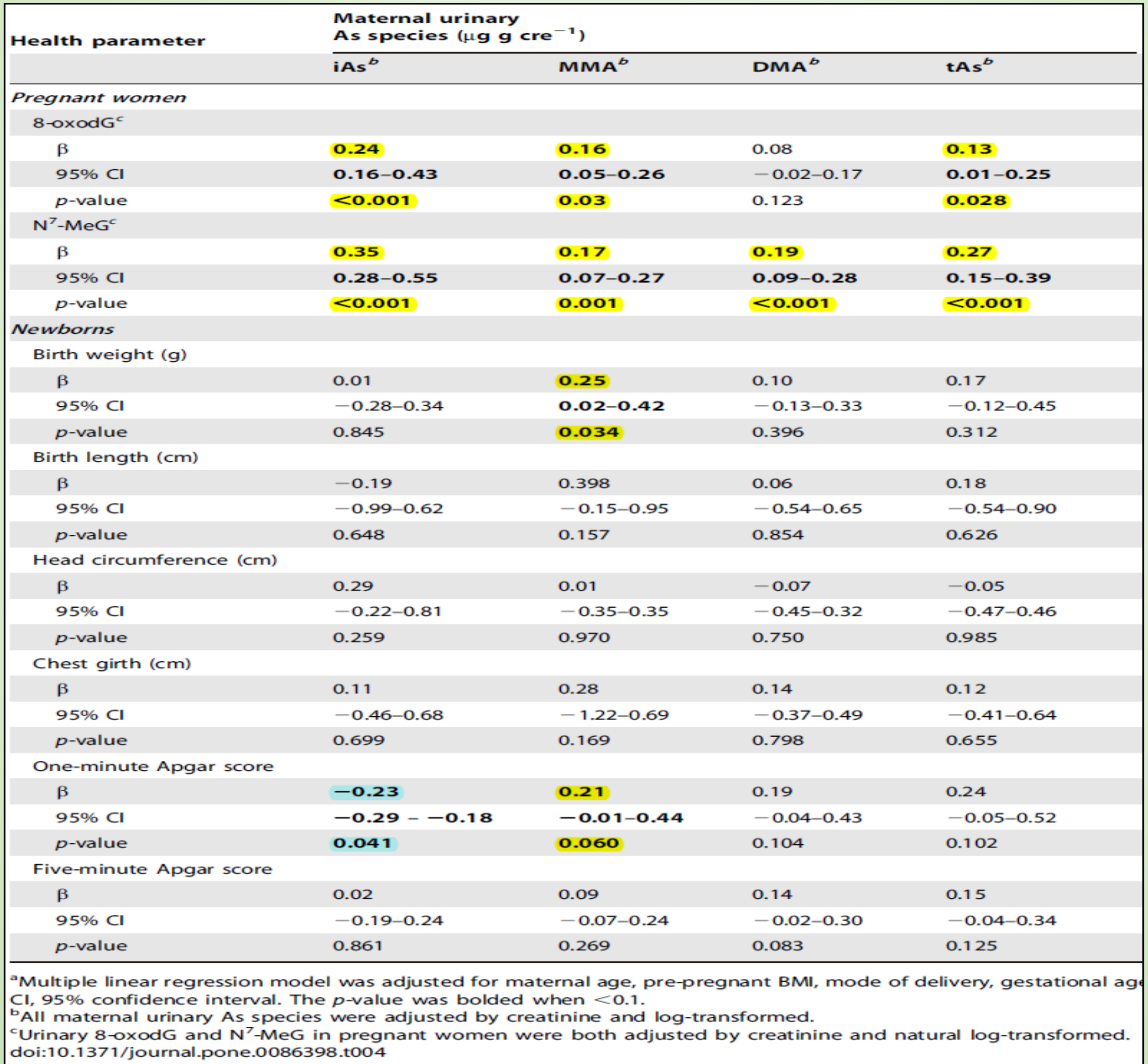


Immune dysfunction



Naujokas et al. EHP 2013

Multiple linear regression (β-coefficient) for the association[a] between birth outcomes with maternal urinary arsenic metabolites[b] and DNA damage[c] biomarkers (n = 299).



Health parameter	Maternal urinary As species (μg g cre^{-1})			
	iAs[b]	MMA[b]	DMA[b]	tAs[b]
Pregnant women				
8-oxodG[c]				
β	**0.24**	**0.16**	0.08	**0.13**
95% CI	**0.16–0.43**	**0.05–0.26**	−0.02–0.17	**0.01–0.25**
p-value	**<0.001**	**0.03**	0.123	**0.028**
N^{7}-MeG[c]				
β	**0.35**	**0.17**	**0.19**	**0.27**
95% CI	**0.28–0.55**	**0.07–0.27**	**0.09–0.28**	**0.15–0.39**
p-value	**<0.001**	**0.001**	**<0.001**	**<0.001**
Newborns				
Birth weight (g)				
β	0.01	**0.25**	0.10	0.17
95% CI	−0.28–0.34	**0.02–0.42**	−0.13–0.33	−0.12–0.45
p-value	0.845	**0.034**	0.396	0.312
Birth length (cm)				
β	−0.19	0.398	0.06	0.18
95% CI	−0.99–0.62	−0.15–0.95	−0.54–0.65	−0.54–0.90
p-value	0.648	0.157	0.854	0.626
Head circumference (cm)				
β	0.29	0.01	−0.07	−0.05
95% CI	−0.22–0.81	−0.35–0.35	−0.45–0.32	−0.47–0.46
p-value	0.259	0.970	0.750	0.985
Chest girth (cm)				
β	0.11	0.28	0.14	0.12
95% CI	−0.46–0.68	−1.22–0.69	−0.37–0.49	−0.41–0.64
p-value	0.699	0.169	0.798	0.655
One-minute Apgar score				
β	**−0.23**	**0.21**	0.19	0.24
95% CI	**−0.29 – −0.18**	**−0.01–0.44**	−0.04–0.43	−0.05–0.52
p-value	**0.041**	**0.060**	0.104	0.102
Five-minute Apgar score				
β	0.02	0.09	0.14	0.15
95% CI	−0.19–0.24	−0.07–0.24	−0.02–0.30	−0.04–0.34
p-value	0.861	0.269	0.083	0.125

[a] Multiple linear regression model was adjusted for maternal age, pre-pregnant BMI, mode of delivery, gestational ag

CI, 95% confidence interval. The *p*-value was bolded when <0.1.

[b] All maternal urinary As species were adjusted by creatinine and log-transformed.

[c] Urinary 8-oxodG and N^{7}-MeG in pregnant women were both adjusted by creatinine and natural log-transformed.

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Kaushal *et al. Environmental Health* (2017) 16:50
DOI 10.1186/s12940-017-0262-0

Environmental Health

RESEARCH

Open Access

Genome-wide DNA methylation at birth in relation to in utero arsenic exposure and the associated health in later life

Akhilesh Kaushal[1], Hongmei Zhang[1*†], Wilfried J. J. Karmaus[1], Todd M. Everson[2], Carmen J. Marsit[2], Margaret R. Karagas[3,4], Shih-Fen Tsai[5], Hui-Ju Wen[5] and Shu-Li Wang[5,6,7,8†]

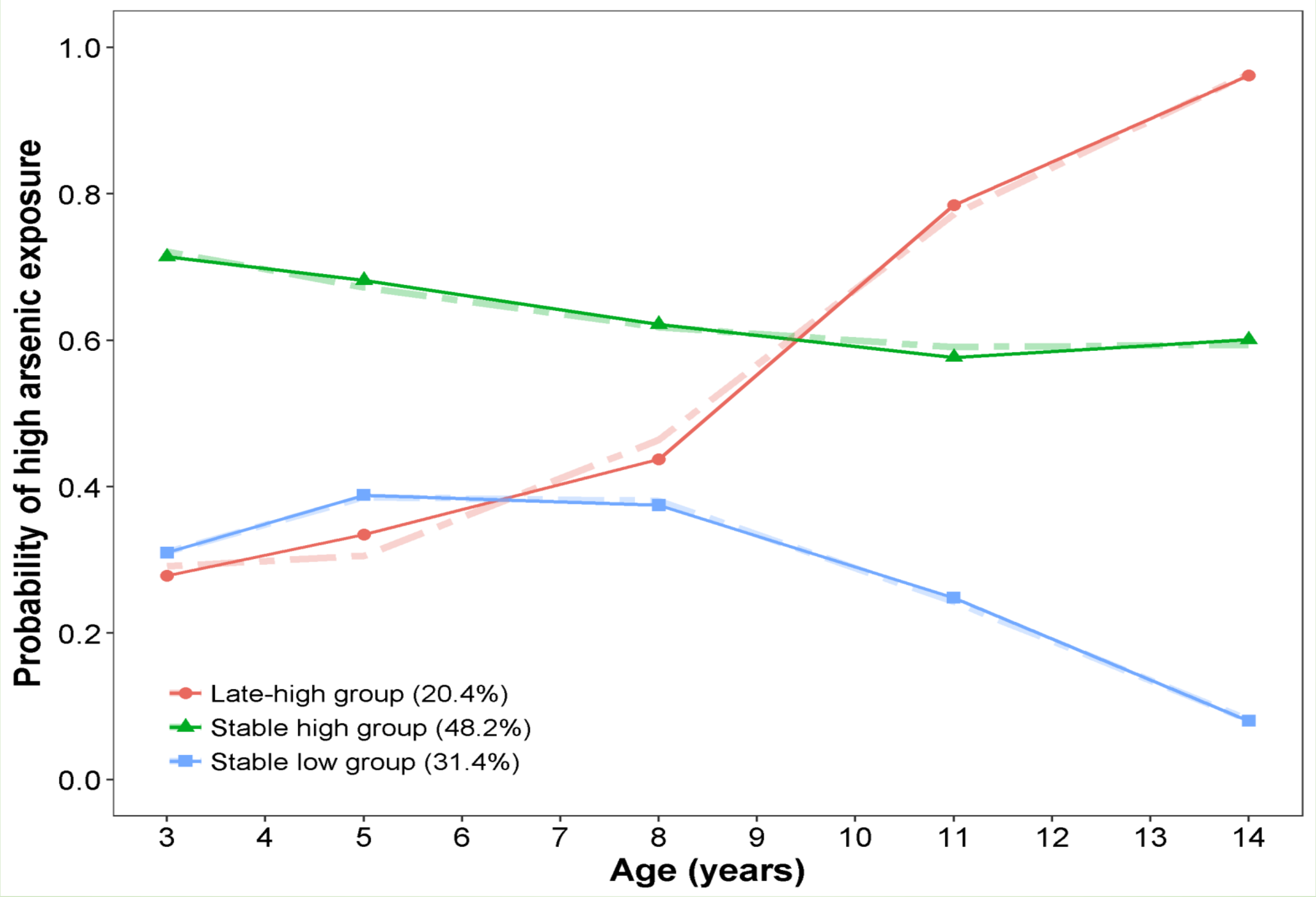
Abstract

Background: In utero arsenic exposure may alter fetal developmental programming by altering DNA methylation, which may result in a higher risk of disease in later life. We evaluated the association between in utero arsenic exposure and DNA methylation (DNAm) in cord blood and its influence in later life.

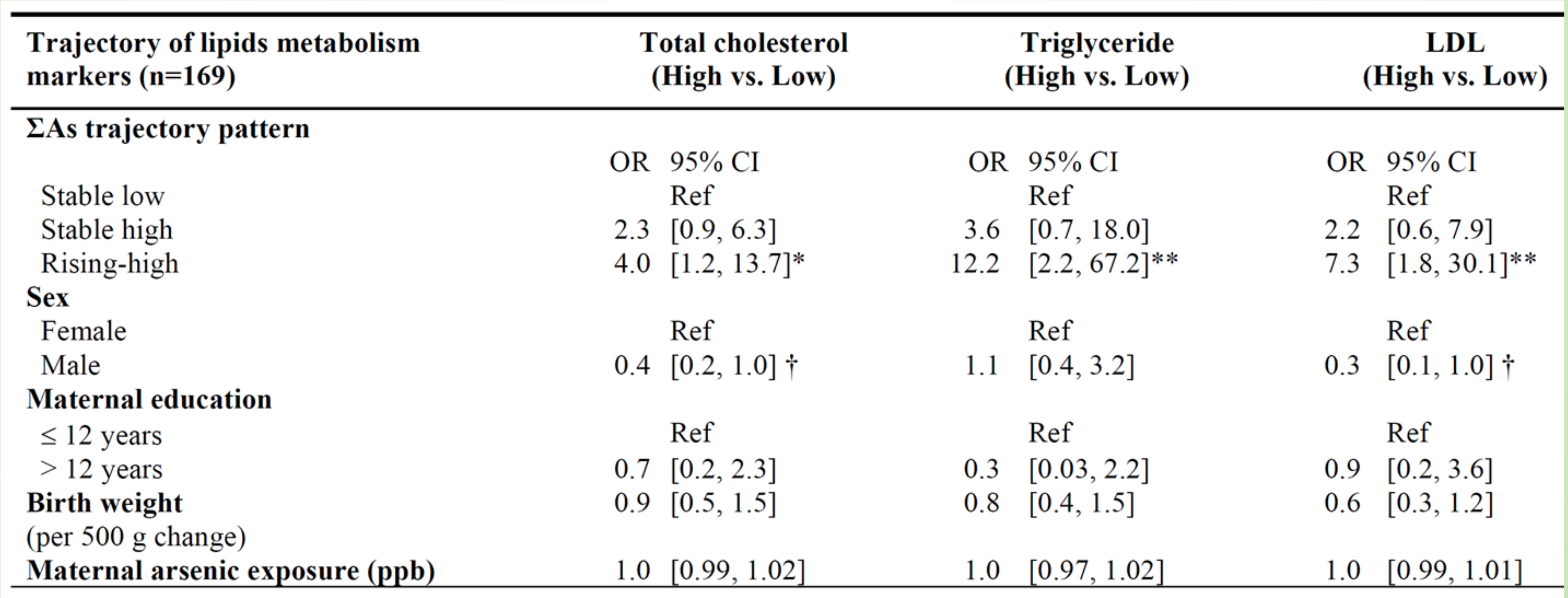
Conclusion: In utero arsenic exposure was associated with cord blood DNA methylation at various CpGs. The identified CpGs may help determine pathological epigenetic mechanisms linked to in utero arsenic exposure. Five CpGs (cg25189764, cg04986899, cg04903360, cg08198265 and cg10473311) may serve as epigenetic markers for changes in LDL later in life.

Keywords: Arsenic, DNA methylation, CpG, DAVID, KEGG pathway, Genome-wide, Ldl

Post-natal arsenic exposure trajectory



Post-natal arsenic exposure trajectory groups and lipid trajectory groups over a 15-year follow-up



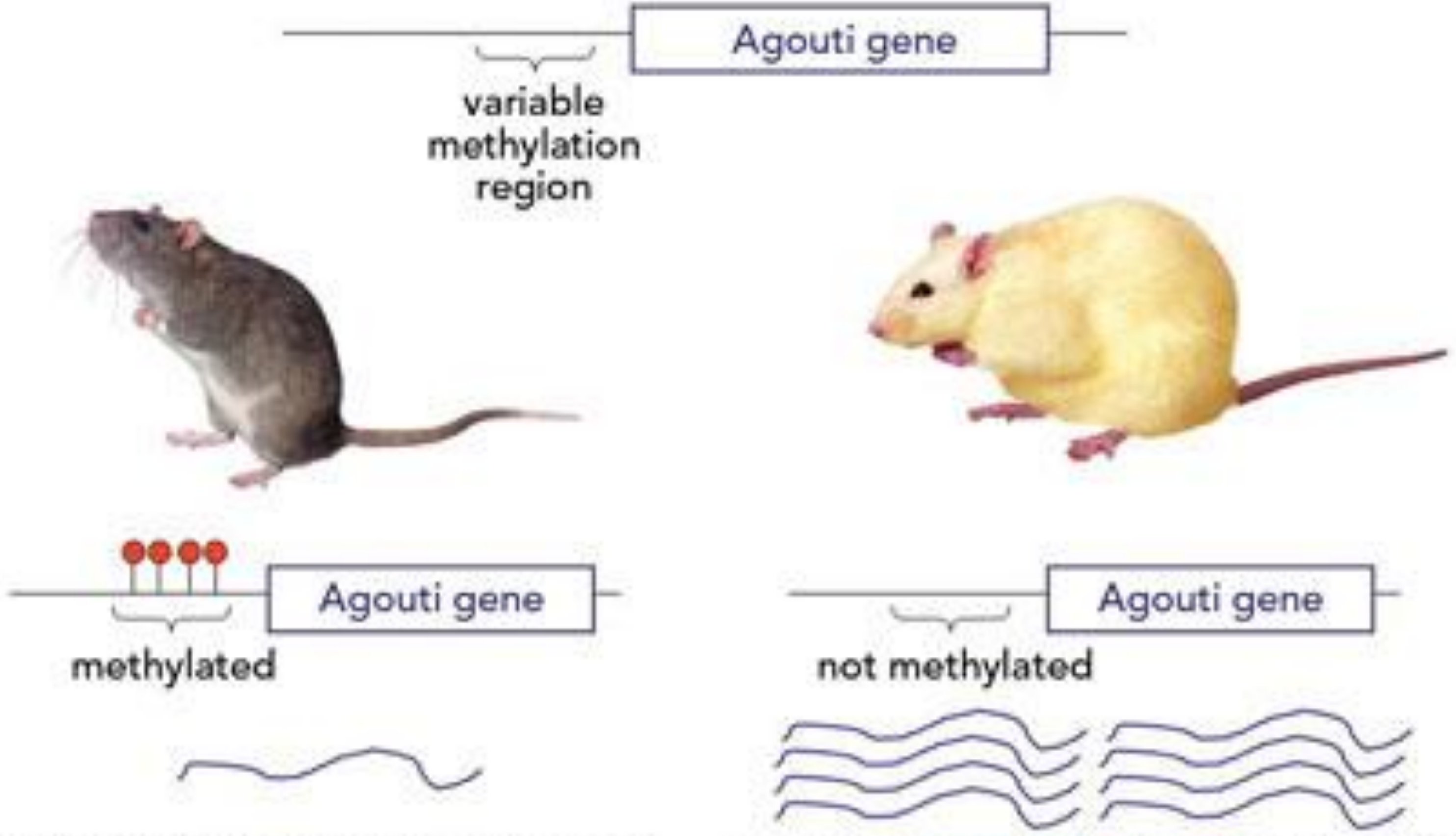
Trajectory of lipids metabolism markers (n=169)	**Total cholesterol (High vs. Low)**		**Triglyceride (High vs. Low)**		**LDL (High vs. Low)**	
ΣAs trajectory pattern						
	OR	95% CI	OR	95% CI	OR	95% CI
Stable low		Ref		Ref		Ref
Stable high	2.3	[0.9, 6.3]	3.6	[0.7, 18.0]	2.2	[0.6, 7.9]
Rising-high	4.0	[1.2, 13.7]*	12.2	[2.2, 67.2]**	7.3	[1.8, 30.1]**
Sex						
Female		Ref		Ref		Ref
Male	0.4	[0.2, 1.0] †	1.1	[0.4, 3.2]	0.3	[0.1, 1.0] †
Maternal education						
≤ 12 years		Ref		Ref		Ref
> 12 years	0.7	[0.2, 2.3]	0.3	[0.03, 2.2]	0.9	[0.2, 3.6]
Birth weight (per 500 g change)	0.9	[0.5, 1.5]	0.8	[0.4, 1.5]	0.6	[0.3, 1.2]
Maternal arsenic exposure (ppb)	1.0	[0.99, 1.02]	1.0	[0.97, 1.02]	1.0	[0.99, 1.01]

Kuo et al. 2018, Environ. Int.

Fetal origins of common diseases

- Fetal origins of cancer, cardiovascular disease, allergies, asthma, hypertension, diabetes, obesity, mental illness — even arthritis, osteoporosis & cognitive decline
- Intrauterine environment is even more important than genes or shared eating habits in passing on a propensity (i.e. obesity, Kral et al.)
- Through epigenetic modification

Altered epigenome by Environment



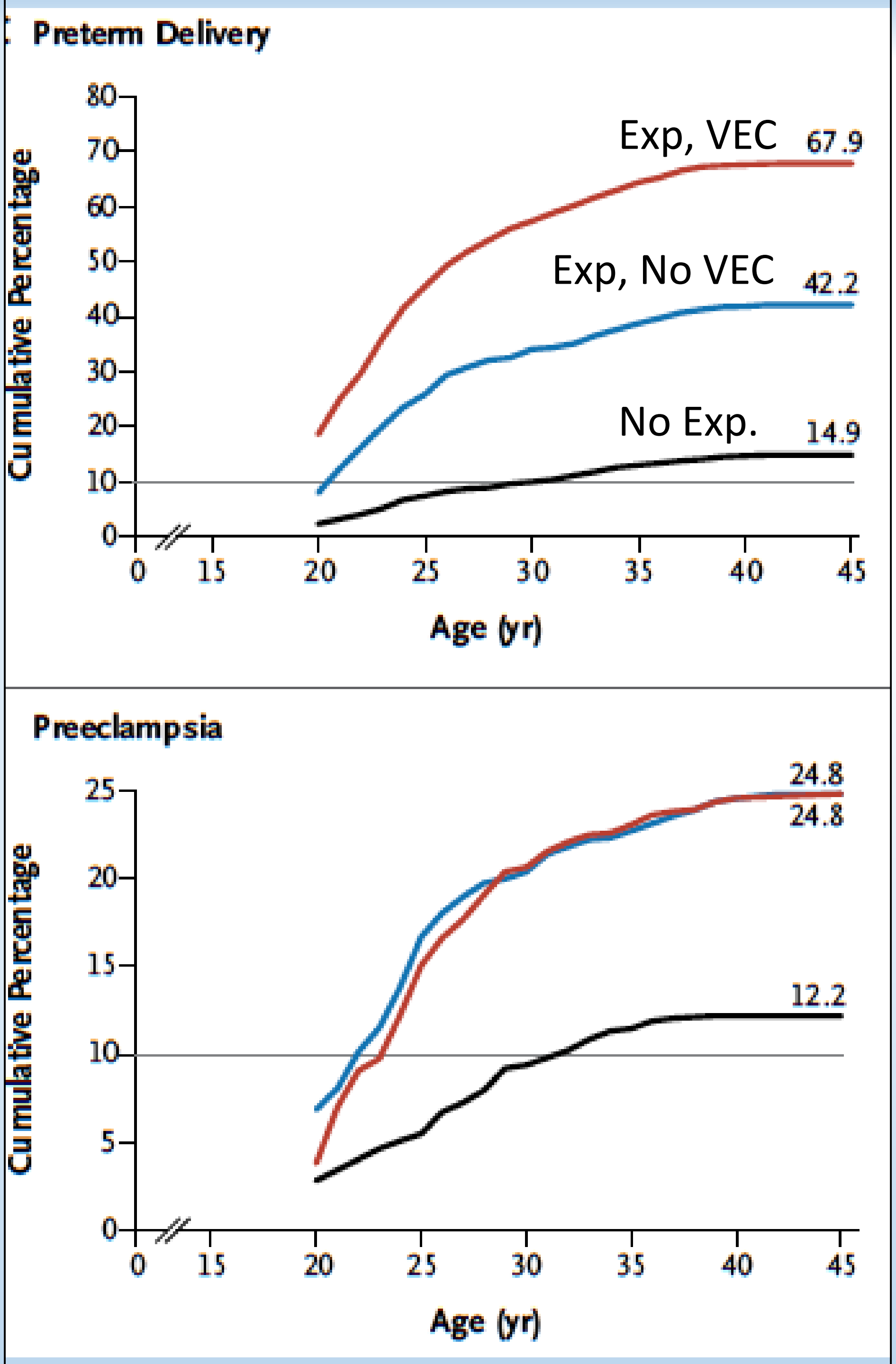
agouti mRNA briefly made during development. agouti gene silenced remainder of mouse life.

agouti gene is continually active, producing mRNA across the mouse lifespan.

healthy mouse with brown fur

mouse with yellow fur;
develops obesity and diabetes during adulthood.

http://www.hudsonalpha.org/education/outreach/basics/epigenetics

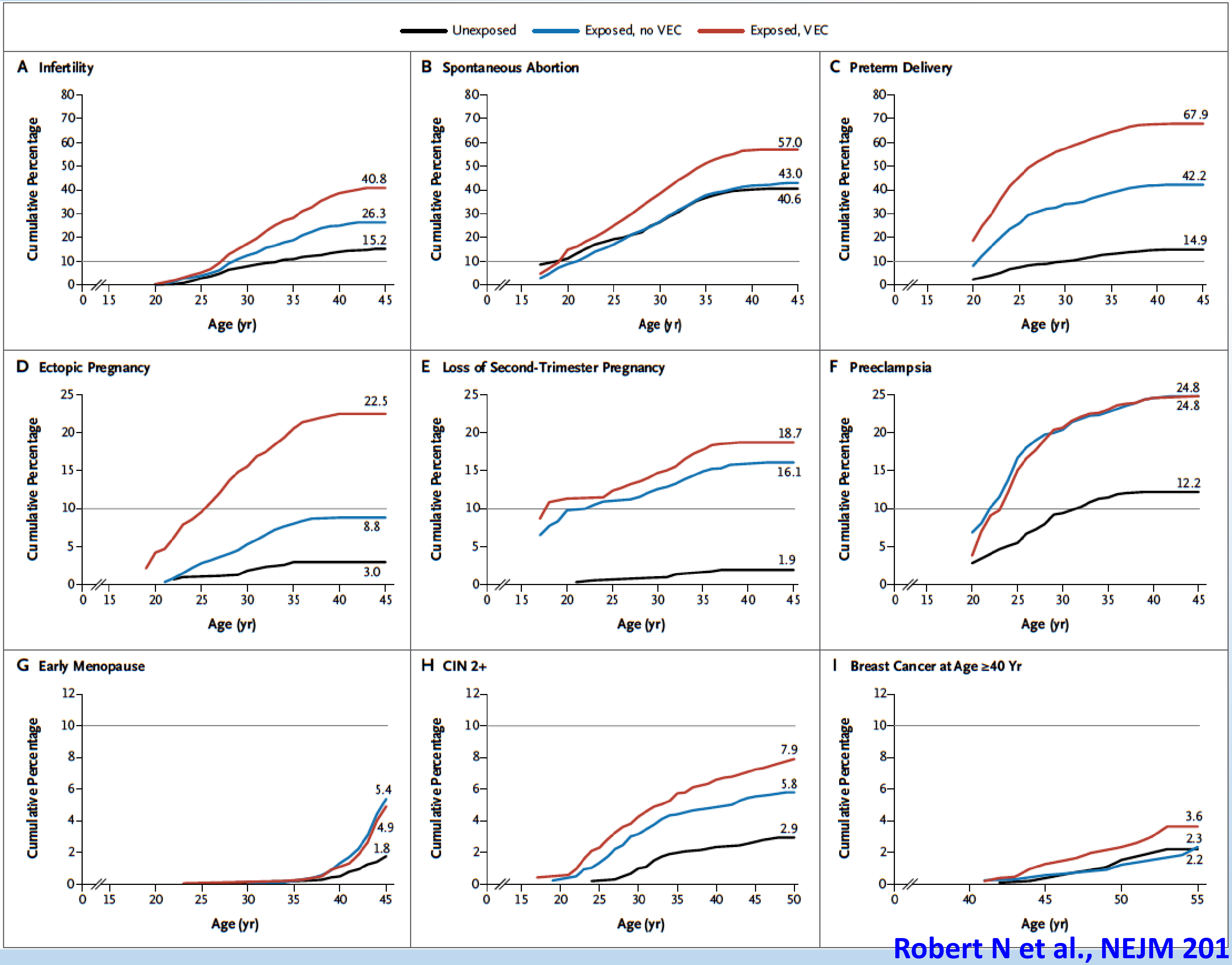


Exp: to diethylstilbestrol (DES)

VEC: Vagina epithelial change

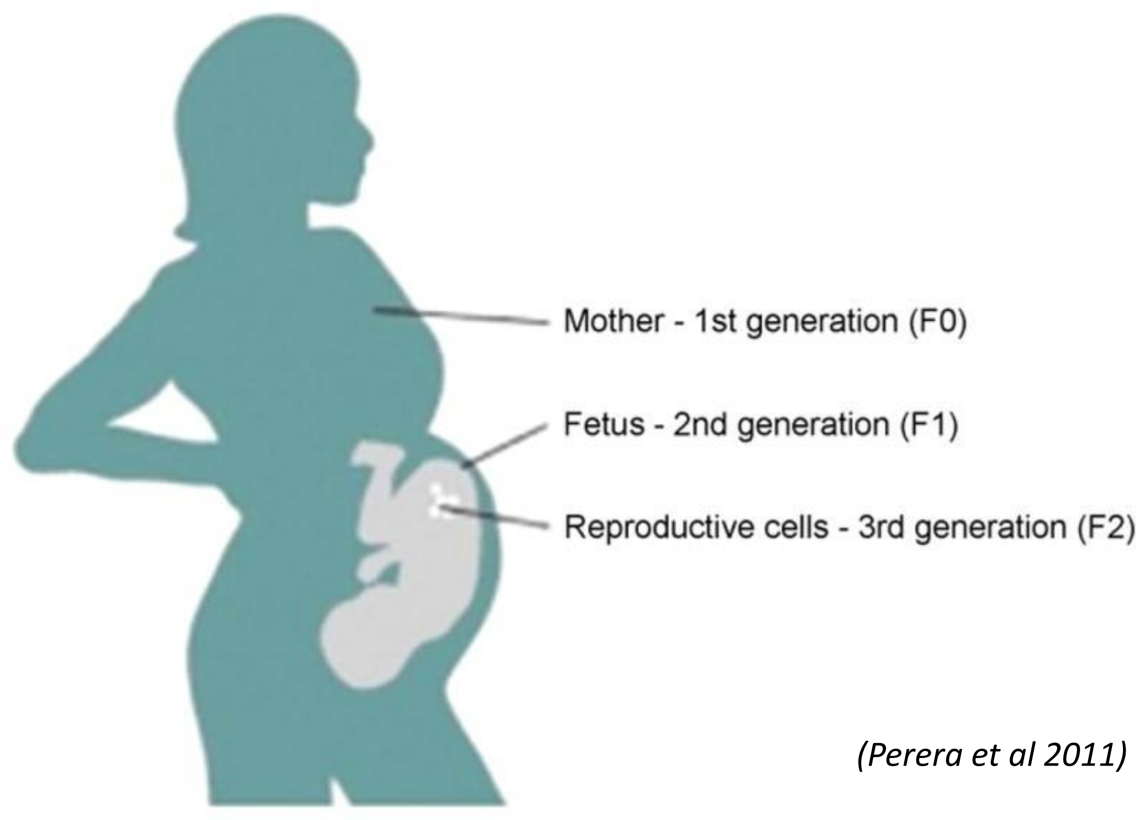
Robert N et al., NEJM 2011

Health Effects in Women Exposed in Utero to DES



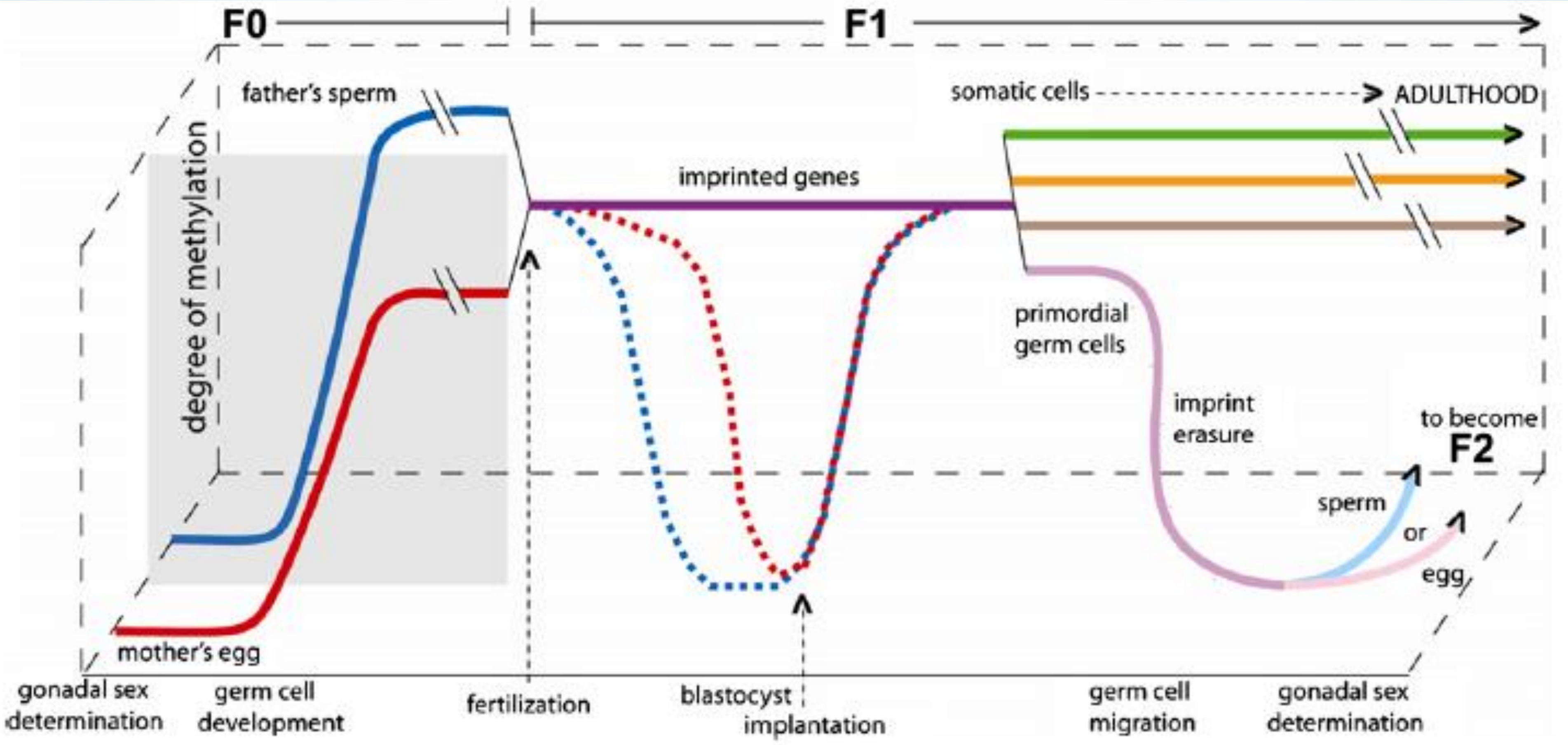
Robert N et al., NEJM 2011

Epigenetic inheritance by observing an epigenetic change in the 4th generation



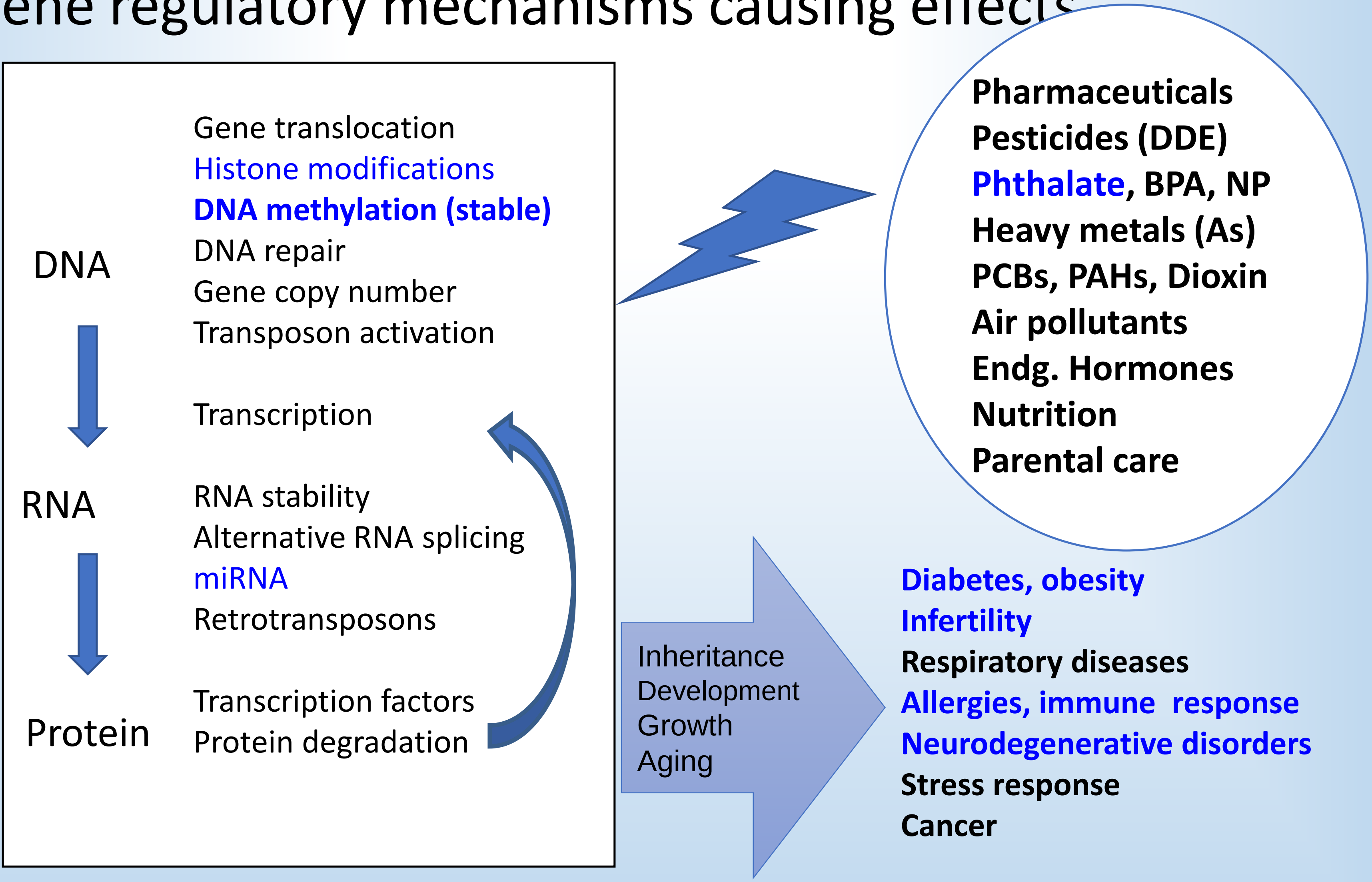
(Perera et al 2011)

DNA Methylation reprograming



(Perera et al 2011)

Selected environmental exposures which also have gene regulatory mechanisms causing effects



DNA

Gene translocation
Histone modifications
DNA methylation (stable)
DNA repair
Gene copy number
Transposon activation

Transcription

RNA

RNA stability
Alternative RNA splicing
miRNA
Retrotransposons

Protein

Transcription factors
Protein degradation

Pharmaceuticals
Pesticides (DDE)
Phthalate, BPA, NP
Heavy metals (As)
PCBs, PAHs, Dioxin
Air pollutants
Endg. Hormones
Nutrition
Parental care

Inheritance
Development
Growth
Aging

Diabetes, obesity
Infertility
Respiratory diseases
Allergies, immune response
Neurodegenerative disorders
Stress response
Cancer

- Non geno toxic
- High plasticity (i.e. body weight)
- Sensitive to the environment (i.e. diet)
  - Altered gene regulations
  - Epigenetics
    - Histone modification (acetylation - relaxed & transcriptionally active DNA)
    - DNA methylation (silencing) - hypomethylated genes *tend* to be more accessible
    - Micro-RNA (suppressed gene expression)
- Endocrine signaling pathways during development predisposition to obesity: EDCs (diethylstilbestrol, bisphenol A, phytoestrogens, phthalates, and organotins, tobacco, PAH)
- Risk assessment and disease prevention

(Perera et al 2011)

Future perspectives

- Early-life environmental factors may have long-term health effects through altered DNA methylations
- Form the Asian consortium on cord blood DNA methylation panel
- Protection of young women and pregnant women
- This deserve big data analysis input for pathway analyses and parallel studies
- Application of precision medicine combining other data on pollutions, heath records, biomarkers, etc.

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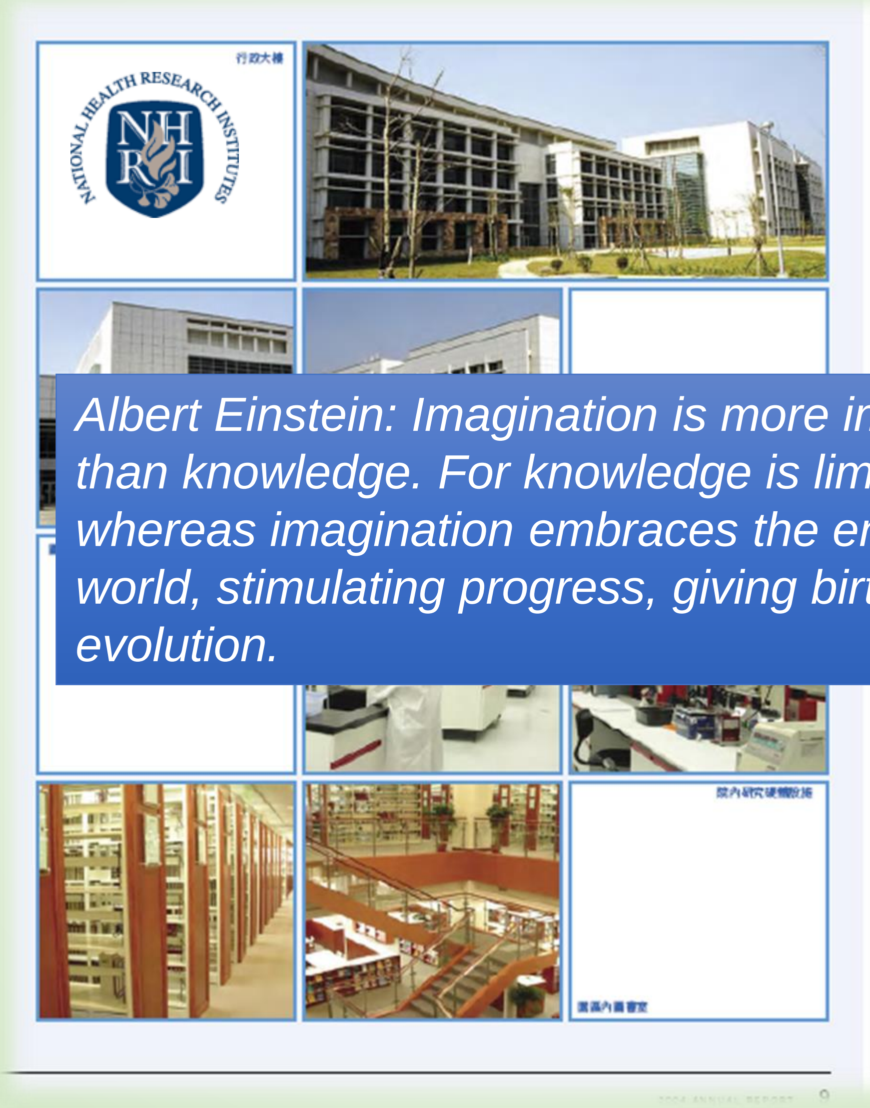

Albert Einstein: Imagination is more important than knowledge. For knowledge is limited, whereas imagination embraces the entire world, stimulating progress, giving birth to evolution.

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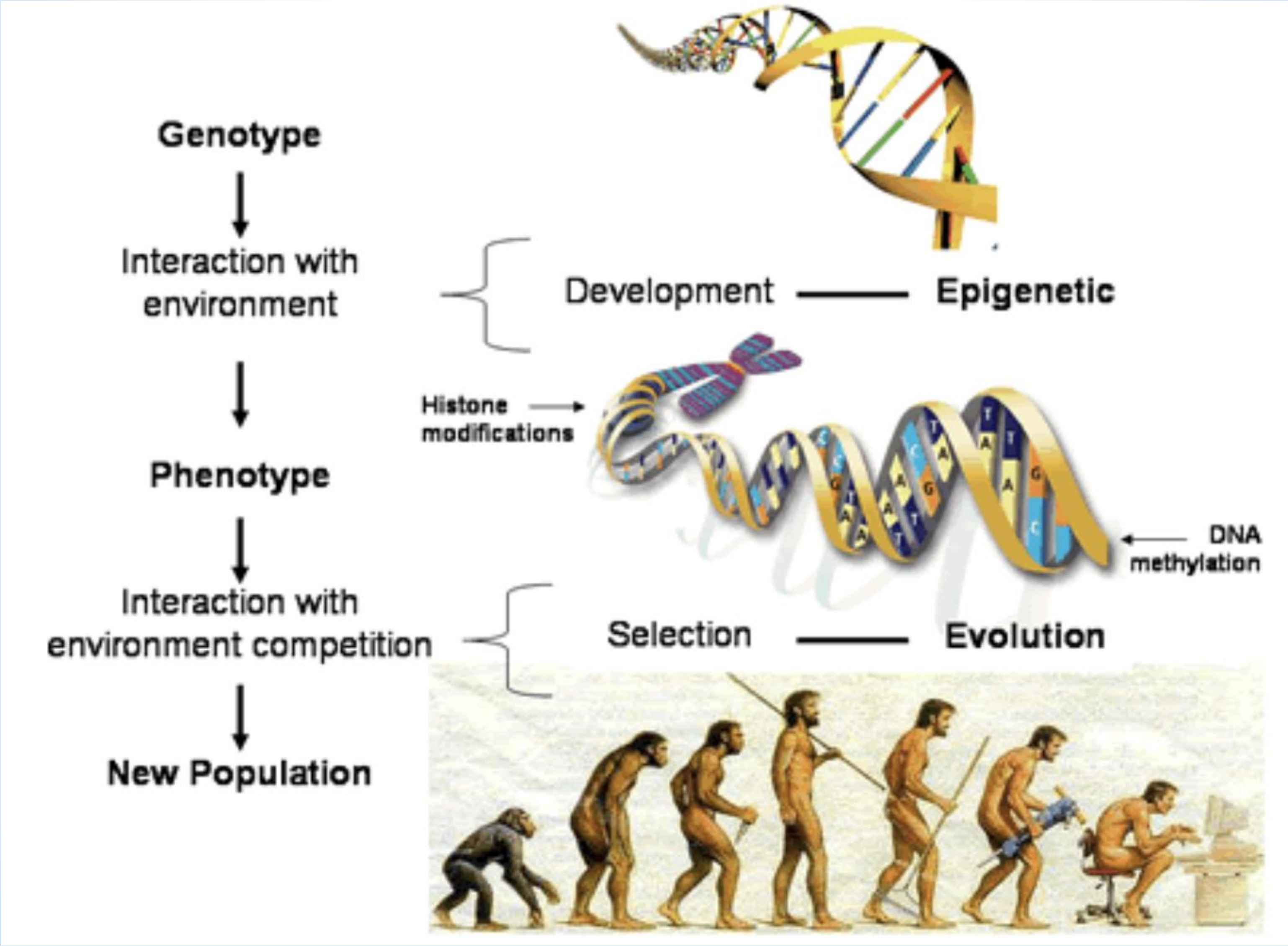
Altered DNA methylation according to Phthalates

- Wnt / β-Catenin Signaling Pathway is active in the biological process with probability 0.976, based on model-based gene set analysis (MGSA) and C2.all in MSigDB.
- This pathway has a role in tumorgenicity.
- CpG sites in six genes in this pathway are simultaneously hypermethylated when MEOHP exposure is higher.

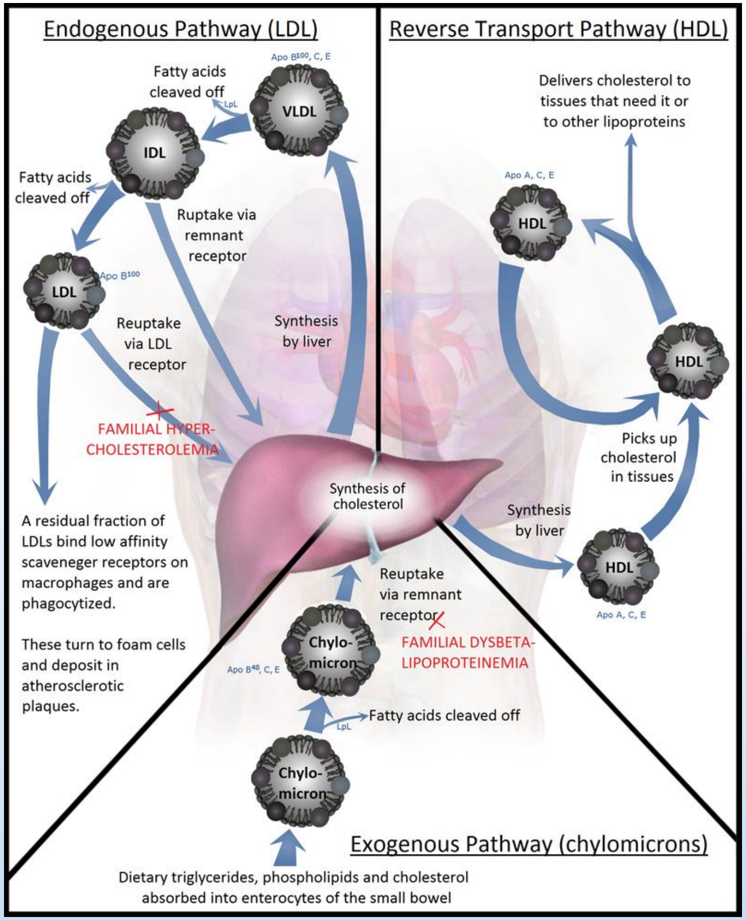
C2 gene set name	inPopulation	inStudySet	estimate	std.error
ST_WNT_BETA_CATENIN_PATHWAY	34	6	0.9759	0.0013
SYMBOL	Coef.	p-value	q-value	
ANKRD6	0.0037	3.30E-06	2.33E-02	
CER1	0.0023	6.25E-05	1.28E-01	
AKT1	0.0018	2.03E-04	2.47E-01	
NKD1	0.0025	2.39E-04	2.72E-01	
DKK3	0.0028	3.98E-04	3.46E-01	
NKD2	0.0025	7.07E-04	4.63E-01	

Chen et al.

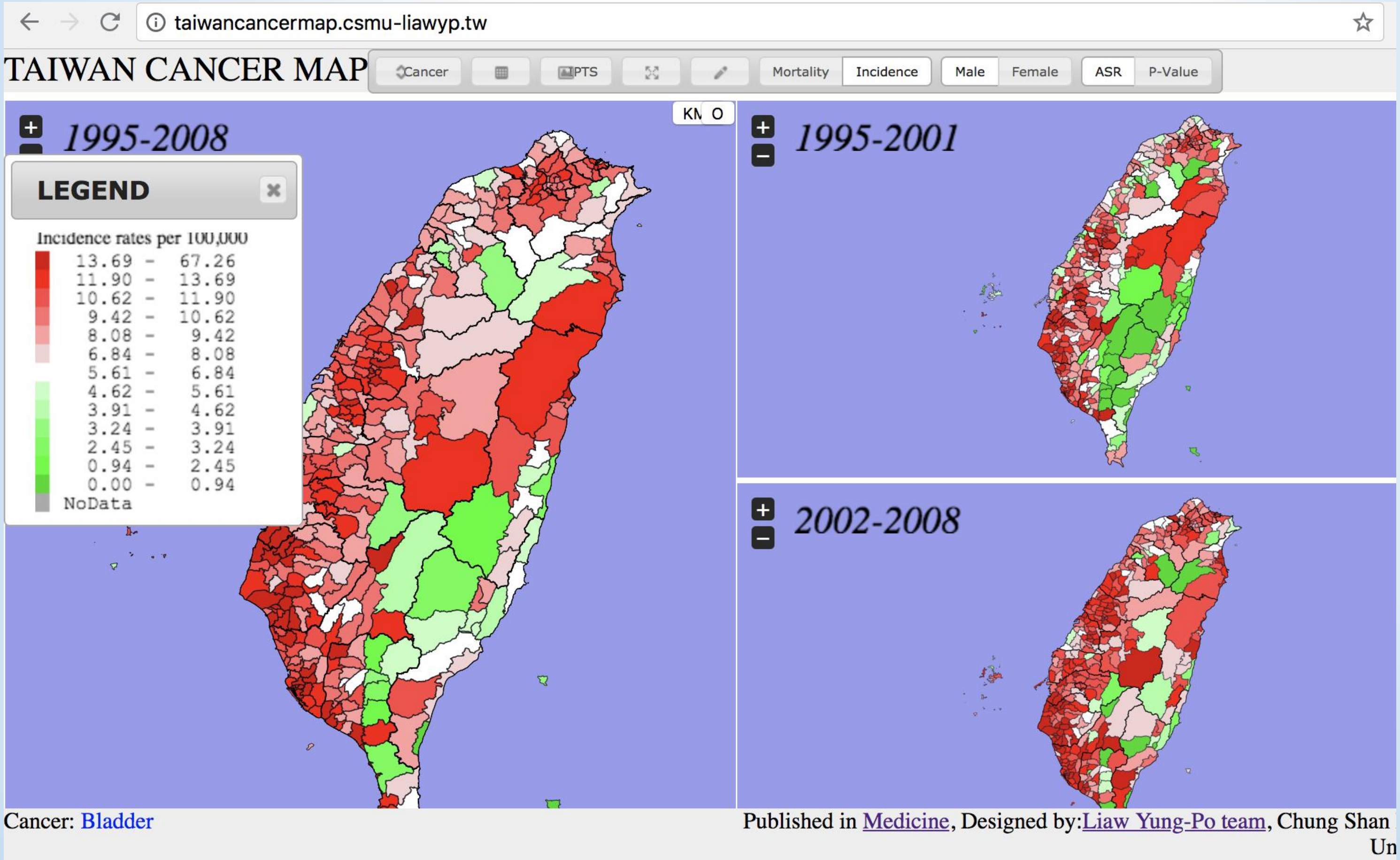
Transgenerational Chx.



“Rising to high” vs. “Stable high”



Bladder Cancer incidence in Taiwan



TAIWAN CANCER MAP

LEGEND

Incidence rates per 100,000

13.69 - 67.26
11.90 - 13.69
10.62 - 11.90
9.42 - 10.62
8.08 - 9.42
6.84 - 8.08
5.61 - 6.84
4.62 - 5.61
3.91 - 4.62
3.24 - 3.91
2.45 - 3.24
0.94 - 2.45
0.00 - 0.94
NoData

Cancer: Bladder

Published in Medicine, Designed by:Liaw Yung-Po team, Chung Shan Un

Why…How…

2007 11 10

Study population I (TMICS – cycle 1)

2012~2014 (n=1524)

- Question.
  - Demog./SES
  - Repro./Med.
  - Life-style
  - Diet habit
- Urine, blood, DNA
  - B-Pb、S-PFASs
  - U-creatinine
  - U- phthalates iAs
  - U- 8-OHdG, N7-MG
  - sIgE
  - B-CVD Chemistry
  - Hormones

2013～2014 (1270)

- Questn.
  - Diet, Growth
- Newborn out.
- AGD measurement.

- Cord Blood, DNA, RNA
- Breast milk
- Placenta
- Meconium
- B-Pb, S-PFAS
- IgE, IgA
- Hormone
- DNA methylation

2003 -2 yr

TMICS
Taiwan Maternal
and Infant Cohort Study

- Question.
- Environ. Exp
- Diet Habit
- HOME
- Bayley II
- Tempmt.
- Activity scale
- Urine, Blood, DNA, RNA
  - U-Phth, B-Pb, U-Creatinine
  - sIgE, Inflammation , cytokines
- Hormone